L2: Entry 1 of 44 File: PGPB Sep 21, 2006

DOCUMENT-IDENTIFIER: US 20060210558 A1
TITLE: Hematopoietic cell selectin ligand polypeptides and methods of use thereof

Description of Disclosure:
[0218] Using the parallel-plate flow chamber under defined hydrodynamic shear stress, L-selectin-mediated tethering and rolling of leukocytes over glutaraldehyde-fixed human HC monolayers was observed. All experiments included negative controls to verify the sole contribution of L-selectin in mediating cell-cell adherence. A shear stress threshold of .about.0.5 dynes/cm.sup.2 was required for L-selectin-mediated adhesive interactions in this system as previously demonstrated (Lawrence, M. B., Kansas, G. S., Kunkel, E. J. and Ley, K. (1997) J. Cell Biol. 136(3), 717-727; Finger, E. B., Puri, K. D., Alon, R., Lawrence, M. B., von Andrian, U. H. and Springer, T. A. (1996) Nature 379(6562):266-269.) (FIG. 6A). After reaching this level of shear stress, leukocyte tethering and rolling was enumerated over a broad shear stress range. L-selectin-dependent human lymphocyte and rat TDL rolling on HL60 cells at a shear stress range of 0.4 dynes/cm.sup.2 to a maximum of 10 dynes/cm.sup.2 was observed (FIG. 6A). However, there was no evidence of lymphocyte rolling on K562 cells, and, despite high PSGL-1 expression, RPMI 8402 cells also did not display L-selectin ligand activity (FIG. 6A and Table 2). In contrast, L-selectin-mediated human lymphocyte and rat TDL rolling on KG1a cell monolayers was observed at shear stress levels in excess of 26 dynes/cm.sup.2, whereas, on HL60 cells, human lymphocyte/TDL rolling was absent past 17 dynes/cm.sup.2 (FIG. 6A). In addition, the frequency of rolling lymphocytes on KG1a cells was up to a 5-fold greater over the entire range of shear stress that supported L-selectin-mediated rolling on HL60 cells (FIG. 6A). The disparity between the high L-selectin ligand activity on KG1a cells and low activity on HL60 cells was also observed by using human neutrophils, which expressed equivalent levels of L-selectin by flow cytometric analysis: KG1a cells supported 4-fold greater L-selectin-mediated neutrophil rolling than that on HL60 cells (FIG. 6B). These data show that KG1a cells possess greater capacity to support L-selectin mediated leukocyte adherence over a broader range of shear stress and that L-selectin natively expressed on lymphocytes or on neutrophils exhibits comparable binding activity to HCELL or to PSGL-1 expressed on human HCs.

Detail Description Paragraph:
[0104] (1) Alon, R., Chen, S., Puri, K. D., Finger, E. B., and Springer, T. A. (1997). The kinetics of L-selectin tethers and the mechanics of selectin-mediated rolling. J. Cell Biol. 138, 1169-1180.

Detail Description Paragraph:
[0113] (10) Chen, S., Alon, R., Fuhlbrigge, R. C., and Springer, T. A. (1997). Rolling and transient tethering of leukocytes on antibodies reveal specializations of selecting. Proc. Natl. Acad. Sci. USA 94, 3172-3177.

Detail Description Paragraph:
[0144] (41) Puri, K. D., Chen, S., and Springer, T. A. (1998). Modifying the mechanical property and shear threshold of L-selectin adhesion independently of equilibrium properties. Nature 392, 930-933.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 5. Document ID: US 20040067544 A1

L3: Entry 5 of 14 File: PGPB Apr 8, 2004

DOCUMENT-IDENTIFIER: US 20040067544 A1
TITLE: Use of adhesion molecules as bond stress-enhanced nanoscale binding switches

Summary of Invention Paragraph:
[0012] Alon, R., et al. (1997) "The kinetics of L-selectin tethers and the mechanics of selectin-mediated rolling," J. Cell Biol. 138:1169-1180.

Summary of Invention Paragraph:
[0034] Finger, E. B. et al. (1996) "Adhesion through L-selectin requires a threshold hydrodynamic shear," Nature 379:266-269.

DOCUMENT-IDENTIFIER: US 6699719 B2
TITLE: Biosensor arrays and methods

Other Reference Publication (9):
Finger, E. B. et al., "Adhesion through L-selectin requires a threshold hydrodynamic shear", Nature, 1996, 379:266-269.

Other Reference Publication (14):
Groves, J. T. et al., "Electrical Manipulation of Glycan-Phosphatidyl Inositol Tethered Proteins in Planar Supported Bilayers", Biophysical Journal, 1996, 71:2716-2723.

Other Reference Publication (28):
Lawrence, M. B. et al., "Leukocytes roll on a selectin at physiological flow rates: distinction from and prerequisite for adhesion through integrins", Cell, 1991, 65:859-873.

Previous

Wu X. and Merchuk J. C. "A model integrating fluid dynamics in photosynthesis and photoinhibition processes," Chem. Eng. Sci. 56:3527-3538, 2001 (hereinafter "Wu and Merchuk, 2001," incorporated herein by reference); Merchuk J. C., et al. "Light-dark cycles in the growth of the red microalga Porphyridium sp.," Biotechnology and Bioengineering, 59:705-713, 1998; Marra, J. "Phytoplankton Photosynthetic Response to Vertical Movement in A Mixed Layer." Mar. Biol. 46:203, 1978)

Wu X. and Merchuk J. "Simulation of Algae Growth in a Bench-Scale Bubble Column Reactor" Biotechnology and Bioengineering, 80:pp. 156-168 (2002)(hereinafter "Wu and Merchuk, 2002")

Wu X. and Merchuk J. "Simulation of algae growth in a bench scale internal loop airlift reactor" Chemical Engineering Science, 59:pp. 2899-2912 (2004)(hereinafter "Wu and Merchuk, 2004");

DOCUMENT-IDENTIFIER: US 20020164748 A1
TITLE: Glycosyl sulfotransferase-3

Detail Description Paragraph:
[0054] GST-3 proteins and polypeptides may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism, such as E. coli, B. subtilis, S. cerevisiae, insect cells in combination with baculovirus vectors, or cells of a higher organism such as vertebrates, particularly mammals, e.g. COS 7 cells, may be used as the expression host cells. In some situations, it is desirable to express the GST-3 gene in eukaryotic cells, where the GST-3 protein will benefit from native folding and post-translational modifications. Small peptides can also be synthesized in the laboratory. Polypeptides that are subsets of the complete GST-3 sequence may be used to identify and investigate parts of the protein important for function.

Detail Description Paragraph:
[0134] Full-length cDNAs containing the two contigs and predicting CS6T/KSST homologs were obtained by screening a human fetal brain .lambda.ZAP cDNA library (Stratagene, La Jolla, Calif.) with labeled 700-800 bp restriction fragments (from EST 2 for contig 1 and from EST 5 for contig 2). Briefly, 10.sup.6 plaque-forming units were used to infect E. coli, which were then distributed on 20 plates. Duplicate filter lifts were performed. The probes were labeled with .sup.32P by random priming (Amersham), and hybridization was performed at 60.degree. C. with high stringency washing. In both screens, multiple positive spots were obtained in the first round. Single positive clones were obtained after either the second or third round of screening. Multiple clones were sequenced for each gene and the presence of the ESTs was confirmed. As will be described below, the cDNAs contain open reading frames that encode predicted proteins of high homology to CS6T/KSST. The proteins encoded by these cDNAs were designated as GST 1 and GST 2, where "GST" denotes "glycosylsulfotransferase." GST 1 has been independently cloned and assigned the name "KSGal6ST) by Fukuta et al., J. Biol. Chem. (1997) 272: 32321-8.

Detail Description Paragraph:
[0144] Each reaction was cycled as follows: hold 4 min @ 94.degree. C., then 35 cycles of [30 sec @ 94.degree. C. followed by 30 sec @ 45.degree. C. followed by 1 min @ 72.degree. C.], then hold 6 min @ 72.degree. C. The entire reactions were then fractionated by standard horizontal agarose (1%) gel electrophoresis. Three bands appearing at positions 2.1, 2.2 and 2.3, were excised and DNA eluted from the gel using the QIAquick PCR purification kit (Qiagen Inc. #28104). Eluted DNA was then subcloned into the TA cloning vector pCR-II (stratagene) and E-coli transformed with recombinant plasmids. For each band eight colonies were expanded, and plasmid DNAs isolated and sequenced using standard dideoxynucleotide chain termination methodology with fluorimetric detection.

Detail Description Paragraph:
[0150] A full length cDNA from the HEC library described in the previous section was cloned as follows. The pool selection procedure described in Bakker et al., J. Biol. Chem. (1997) 272:29942-6) was used to quickly isolate the cDNA. It was first established that the relevant template was contained within the library by successfully amplifying the above described PCR product from the library stock comprising the entire library. An aliquot of this bacterial stock was then divided into 200 pools of 2000-3000 colonies each. Each pool was plated out on LB plates and the colonies were allowed to grow to a healthy size. The colonies were harvested in LB and allowed to grow further at 37.degree. C., at which time glycerol stocks were prepared from each pool. By PCR analysis of the pools, nine positives were identified in this first round of screening. The corresponding bacterial stock for one of these pools was then titered and plated at 100 colony forming units (cfu) per plate in 40 plates. Plates were grown,

harvested, preserved and analyzed as in the first round, resulting in the identification of three positive subpools. At this stage, one of the three positive pools was plated at a density (300 cfu) so that individual colonies could be analyzed by PCR. One cDNA clone was obtained by this approach. It contains a complete open reading frame which encodes a novel 386 amino acid protein, termed GST-3. This full length cDNA sequence was then used as template in a BLASTN search of the public (dbest) and Lifeseq EST databases. In this manner, two so far unrecognized ESTs #2617407 (from Lifeseq; derived from a human gall bladder cDNA library) and g2262929 (from the mouse EST collection included in the dbest database, derived from a murine mammary gland cDNA library) were identified. The former EST included the 5' end of GST-3 open reading frame. Since this EST was generated with an oligo dT-primer, it therefore contains the entire open reading frame plus all 3' untranslated sequence of the human GST-3 cDNA. This EST was retrieved from Incyte in the form of a plasmid-transformed E. coli culture, expanded into Luria Bertoni Medium (with 0.1 mg/ml Ampicillin). The plasmid was isolated from a 500 ml culture and sequenced using standard dideoxynucleotide chain termination methodology with fluorimetric detection. Since no PCR-step was used in generating the full length GST-3 Lifeseq EST Incyte #2167407 (in contrast to the Cap-finder methodology employed in generation of our HEV-library), the GST-3 sequence obtained from Incyte #2617407 is free of PCR errors. The sequence is provided in SEQ ID NO: 02 and shown in FIG. 1.

Detail Description Paragraph:
[0181] To determine the contribution of sulfation to tethering under laminar flow conditions, Jurkat cells were perfused through a parallel wall flow chamber in which fucosylated GlyCAM-1/IgG constructs with or without sulfate modifications were coated at the same site densities. Jurkat cells showed the tethering profile characteristic for L-selectin with a shear threshold below which no or little tethering occurred (FIG. 12). While a maximum tethering rate of 9% was found for the interaction of cells with non-sulfated GlyCAM-1/IgG (FT), the fraction of tethered cells was doubled upon sulfation on C-6 of GlcNAc (FT, huGlcNAc6ST; FT, HEC-GlcNac6ST) and increased sixfold upon sulfation C-6 of Gal (FT, KSGal6ST). Furthermore, the latter modification resulted also in a shift of the tethering threshold towards lower shear stresses (FIG. 12).

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☐	L10	L9 and l1	8
☐	L11	L10 not l3	5
☐	L12	l1 and (biofilm or bio-film or cathet$)	18

END OF SEARCH HISTORY